

# APPENDIX A

## EXEMPLARY SUPPORT<sup>1</sup> IN THE PATTEN '221, '671 AND '069 APPLICATIONS FOR CLAIM 275 AS CURRENTLY PENDING

<u>Claim</u>	<u>Exemplary Support</u>
275. A method of producing a library	p. 2, l. 24 – p. 3, l. 5, Claim 16 (“a method...to generate a library...”); p. 16, ll. 18-20 (“starting DNA segments are recombined...to generate a diverse library of recombinant DNA segments”)
comprised of chimerized	p. 31, ll. 32-35 (“nucleic acids encoding protein modules can be exchanged...to generate novel and functional chimeric polyketides”); p. 37, ll. 20-21 (“A library of 10 <sup>4</sup> chimeric interferon genes...”);
but defined polynucleotide sequences	Claim 16 (“a first and second substrate molecules ...comprise defined segments”)
each of which is comprised of a defined number of polynucleotide segments	Claim 16 (“a first and second substrate molecules ...comprise defined segments”); p. 32, l. 6 – p. 34, l. 9 (e.g., “assemble multiple segments”); Example III, p. 89, l. 36 – p. 93, l. 9 (e.g., “The modeled structure...has been divided into nine segments based on a combination of criteria of maintaining secondary structure elements as single units and placing/choosing placement of the segment boundaries in regions of high identity.”)
that are assembled in an ordered fashion,	p. 33, l. 12 (“are reassembled in an ordered fashion...”)
the method comprising:	p. 2, l. 29 (“the method comprising...”)
a) generating a plurality of defined polynucleotide segments of substrate nucleic acid sequences	p. 29, ll. 22-27 (“The coarse grain methods allow one to exchange chunks of genetic material between substrate nucleic acids thereby limiting diversity in the resulting recombinants to exchanges or substitutions of domains, restriction fragments, oligo-encoded blocks of mutations, or other arbitrarily defined segments...”); p. 32, ll. 9-11 (“multiple segments that have been separately evolved...”); p. 32, ll. 17-20 (“Boundaries defining segments of a nucleic acid sequence of interest...”)

<sup>1</sup>The identified support is merely exemplary, and is not meant to be exhaustive. Applicant reserves the right to cite additional support at a later time, if necessary.

APPENDIX A

wherein said substrate nucleic acids encode full-length enzymes,	p. 43, ll. 18-20 ("this technique can be used to evolve bovine intestinal alkaline phosphatase (BIAP)..."; p. 82, ll. 16-25 ("Evolution of BIAP...the oligonucleotides are assembled into full-length genes as described above."); p. 16, ll. 22-24 ("In general, the starting segments and the recombinant libraries generated include full-length coding sequences..."))
and wherein borders defining the polynucleotide segments are selected by aligning the substrate nucleic acid sequences; and	p. 32, ll. 17-20 ("Boundaries defining segments of a nucleic acid sequence of interest preferably lie in intergenic regions, introns, or areas of a gene not likely to have mutations of interest; p. 38, ll. 23-28 ("This region, which can be part or all of a gene or a gene is arbitrarily delineated into segments. The segment borders can be chosen randomly, based on correspondence with natural exons, based on structural considerations (loops, alpha helices, subdomains, whole domains, hydrophobic core, surface, dynamic simulations), and based on correlations with genetic mapping data.)
b) reassembling said defined polynucleotide segments in order	p. 33, l. 12 ("reassembled in an ordered fashion")
thereby producing the library of chimerized but defined polynucleotide sequences,	p. 31, ll. 32-35 ("nucleic acids encoding protein modules can be exchanged...to generate novel and functional chimeric polyketides"); p. 37, ll. 20-21 ("A library of 10 <sup>4</sup> chimeric interferon genes..."); Claim 16 ("a first and second substrate molecules ...comprise defined segments")
such that said segments are reassembled in an ordered fashion	p. 33, l. 12 ("reassembled in an ordered fashion")
to produce each chimerized but defined polynucleotide sequence encoding a full-length enzyme.	p. 31, ll. 32-35 ("nucleic acids encoding protein modules can be exchanged...to generate novel and functional chimeric polyketides"); p. 37, ll. 20-21 ("A library of 10 <sup>4</sup> chimeric interferon genes..."); Claim 16 ("a first and second substrate molecules ...comprise defined segments")

**APPENDIX B**

**THE PROPOSED COUNT**

<b>Claim 6 of the Short '449 patent (incorporating the limitations of Claim 1, from which it depends)</b>	<b>OR</b>	<b>Claim 275 of the Patten '221 application</b>
<p>A method of producing a progeny library comprised of chimerized but pre-determined polynucleotide sequences each of which is comprised of a pre-determined number of building block sequences that are assembled in non-random order, the method comprising:</p> <p>generating a plurality of pre-determined nucleic acid building block sequences obtained from polynucleotide sequences that encode enzymes or fragments thereof and comprised of sequences delineated by demarcation points selected from aligned progenitor sequences; and</p> <p>non-stochastically reassembling said nucleic acid building block sequences to produce said chimerized but pre-determined polynucleotide sequences, such that a designed overall assembly order is achieved for each of said chimerized but pre-determined polynucleotide sequence.</p>		<p>A method of producing a library comprised of chimerized but defined polynucleotide sequences each of which is comprised of a defined number of polynucleotide segments that are assembled in an ordered fashion, the method comprising:</p> <p>a) generating a plurality of defined polynucleotide segments of substrate nucleic acid sequences wherein said substrate nucleic acids encode full-length enzymes, and wherein borders defining the polynucleotide segments are selected by aligning the substrate nucleic acid sequences; and</p> <p>b) reassembling said defined polynucleotide segments in order thereby producing the library of chimerized but defined polynucleotide sequences, such that said segments are reassembled in an ordered fashion to produce each chimerized but defined polynucleotide sequence encoding a full-length enzyme.</p>

**APPENDIX C**

**CLAIMS OF THE SHORT '449 PATENT THAT CORRESPOND WITH THE  
PROPOSED COUNT COMPARED WITH THE PROPOSED COUNT**

<b>Short '449 Patent Claim</b>	<b>Comparison with the proposed Count</b>
<p>1. A method of producing a progeny library comprised of chimerized but pre-determined polynucleotide sequences each of which is comprised of a pre-determined number of building block sequences that are assembled in non-random order, the method comprising:</p> <p>(a) generating a plurality of pre-determined nucleic acid building block sequences comprised of sequences delineated by demarcation points selected from aligned progenitor nucleic acid sequences; and</p> <p>(b) non-stochastically assembling said nucleic acid building block sequences to produce said chimerized but pre-determined polynucleotide sequences, such that a designed overall assembly order is achieved for each of said chimerized but pre-determined polynucleotide sequence.</p>	<p>Claim 6, which is dependent from, and incorporates all the limitations of Claim 1, is one alternative of the Count. As such, Claim 1 is anticipated by the Count, and should correspond thereto.</p>

APPENDIX C

Short '449 Patent Claim	Comparison with the proposed Count
2. The method of claim 1 where the progenitor nucleic acid sequences comprise sequences derived from an uncultivated organism or an environmental sample.	Claim 6, which is dependent from, and incorporates the limitations of Claim 2, is one alternative of the Count. As such, Claim 2 is anticipated by the Count, and should correspond thereto. Moreover, deriving sequences from an uncultivated organism or an environmental sample would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g., Brennan (1996) Chemical and Eng. News 74:31-33</i>
3. The method of claim 1 where the progenitor nucleic acid sequences are comprised of genomic nucleic acid sequences.	Claim 6, which is dependent from, and incorporates the limitations of Claim 3, is one alternative of the Count. As such, Claim 3 is anticipated by the Count, and should correspond thereto. Moreover, starting with genomic sequences is a mere design choice, which would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g., WO 98/27230<sup>1</sup> at p. 36, ll. 29-31</i> {"The starting exon DNA may be synthesized de novo from sequence information, or may be present in any nucleic acid preparation (e.g., genomic, cDNA, libraries, and so on)."} }

<sup>1</sup> Note that WO 98/27230 is the 1998 publication of the PCT application corresponding to the present Patten '221 application specification.

# APPENDIX C

Short '449 Patent Claim	Comparison with the proposed Count
<p>4. The method of claim 1, where the progeny library is comprised of at least <math>10^{10}</math> different pre-determined progeny molecular sequences.</p>	<p>Claim 6, which is dependent from, and incorporates the limitations of Claim 4, is one alternative of the Count. As such, Claim 4 is anticipated by the Count, and should correspond thereto. Moreover, progeny library is comprised of at least <math>10^{10}</math> different pre-determined progeny molecular sequences would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i>, WO 98/27230 at p. 16, ll. 18-22 and p. 91, ll. 26-28 {"The starting DNA segments are recombined by any of the recursive sequence recombination formats provided herein to generate a diverse library of recombinant DNA segments. Such a library can vary widely in size from having fewer than 10 to more than <math>10^5</math>, <math>10^9</math>, or <math>10^{12}</math> members." "Thus, the potential diversity encoded by permutation of all of this naturally occurring diversity is: <math>2^{57} \times 3^{15} \times 4^4 = 5.3 \times 10^{26}</math>"}</p>

APPENDIX C

Short '449 Patent Claim	Comparison with the proposed Count
<p>5. The method of claim 1, where the progeny library is comprised of at least <math>10^{15}</math> different pre-determined progeny molecular sequences.</p>	<p>Claim 6, which is dependent from, and incorporates the limitations of Claim 5, is one alternative of the Count. As such, Claim 5 is anticipated by the Count, and should correspond thereto. Moreover, progeny library is comprised of at least <math>10^{10}</math> different pre-determined progeny molecular sequences would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i>, WO 98/27230 at p. 16, ll. 18-22 and p. 91, ll. 26-28{"The starting DNA segments are recombined by any of the recursive sequence recombination formats provided herein to generate a diverse library of recombinant DNA segments. Such a library can vary widely in size from having fewer than 10 to more than <math>10^5</math>, <math>10^9</math>, or <math>10^{12}</math> members." "Thus, the potential diversity encoded by permutation of all of this naturally occurring diversity is: <math>2^{57} \times 3^{15} \times 4^4 = 5.3 \times 10^{26}</math>"}</p>
<p>6. The method of any of claims 1-5, where the nucleic acid building block sequences are obtained from polynucleotide sequences that encode enzymes or fragments thereof.</p>	<p>Claim 6 is one alternative of the Count.</p>

**APPENDIX C**

Short '449 Patent Claim	Comparison with the proposed Count
<p>7. The method of any of claims 1-5, where the nucleic acid building block sequences are assembled to produce polynucleotide encoding biochemical pathways from one or more operons or gene clusters of portions thereof.</p>	<p>Claim 7 incorporates the limitations of Claim 1, which is obvious in view of the Count. Moreover, assembling building block sequences to produce polynucleotide encoding biochemical pathways from one or more operons or gene clusters of portions thereof would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i>, WO 98/27230 at p. 13, ll. 9-13 {"For example, coarse grain searches are often better suited for optimizing multigene clusters such as polyketide operons, whereas fine grain searches are often optimal for optimizing a property such as protein expression using codon usage libraries."}</p>
<p>8. The method of any of claims 1-5, where the nucleic acid building block sequences are obtained from polynucleotide encoding polyketides or fragments thereof.</p>	<p>Claim 8 incorporates the limitations of Claim 1, which is obvious in view of the Count. Moreover, assembling building block sequences obtained from polynucleotides encoding polyketides or fragments thereof would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i>, WO 98/27230 at p. 13, ll. 9-13 {"For example, coarse grain searches are often better suited for optimizing multigene clusters such as polyketide operons, whereas fine grain searches are often optimal for optimizing a property such as protein expression using codon usage libraries."}</p>

APPENDIX C

Short '449 Patent Claim	Comparison with the proposed Count
9. The method of any of claims 1-5, where the nucleic acid building block sequences are obtained from polynucleotide encoding antibodies or antibody fragments or other peptides or polypeptides.	Claim 9 incorporates the limitations of Claim 1, which is obvious in view of the Count. Moreover, obtaining building block sequences from an antibody would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i> , WO 98/27230, p. 40, ll. 35-37 {"For example, this format is preferred for the in vivo affinity maturation of an antibody by RSR."}
10. The method of any of claims 1-5, where the step of (b) non-stochastically assembling said nucleic acid building blocks is performed to generate a display library comprised of polypeptides or antibodies or peptidomimetic antibodies or antibody variable region sequences suitable for affinity interaction screening.	Claim 10 incorporates the limitations of Claim 1, which is obvious in view of the Count. Moreover, generating a display library comprised of antibody regions would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i> , WO 98/27230, p. 66, ll. 1-2 {"For example, the affinity of an antibody for a ligand can be improved using mammalian surface display and RSR."}
11. The method of any of claims 1-5, further comprising the step of  (c) screening said progeny library to identify an evolved molecular property.	Claim 11 incorporates the limitations of Claim 1, which is obvious in view of the Count. Moreover, screening for an evolved molecular property would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i> , WO 98/27230, p. 8, ll. 31-33 {"A further aspect of the invention is a method for screening a library of protease mutants to obtain an improved protease..."}

**APPENDIX C**

<b>Short '449 Patent Claim</b>	<b>Comparison with the proposed Count</b>
12. The method of claim 1, where step of (c) is comprised of expression screening to identify an evolved molecular property.	Claim 12 incorporates the limitations of Claim 1, which is obvious in view of the Count. Moreover, expression screening to identify an evolved molecular property would have been obvious to one of ordinary skill in the art at the time the application for the '449 patent was filed. <i>See, e.g.</i> , WO 98/27230, p. 9, ll. 32-34 {"A further aspect of the invention is a method for screening a library of mutants of a DNA substrate encoding a protein for an evolved DNA substrate, comprising: (a) providing a library of mutants, the library comprising an expression vector; (b) transfecting a mammalian host cell with the library of (a), wherein mutant protein is expressed on the surface of the cell; (c) screening or selecting the products of (b) with a ligand for the protein..."}

**APPENDIX D**

**PENDING CLAIM 275 OF THE PATTEN '221 APPLICATION THAT  
CORRESPONDS WITH THE PROPOSED COUNT COMPARED WITH THE  
PROPOSED COUNT**

<b>Patten '221 Application Claim</b>	<b>Comparison with the proposed Count</b>
<p>275. A method of producing a library comprised of chimerized but defined polynucleotide sequences each of which is comprised of a defined number of polynucleotide segments that are assembled in an ordered fashion, the method comprising:</p> <p style="padding-left: 40px;">a) generating a plurality of defined polynucleotide segments of substrate nucleic acid sequences wherein said substrate nucleic acids encode full-length enzymes, and wherein borders defining the polynucleotide segments are selected by aligning the substrate nucleic acid sequences; and</p> <p style="padding-left: 40px;">b) reassembling said defined polynucleotide segments in order thereby producing the library of chimerized but defined polynucleotide sequences, such that said segments are reassembled in an ordered fashion to produce each chimerized but defined polynucleotide sequence encoding a full-length enzyme.</p>	<p>Claim 275 is one alternative of the Count</p>

Attorney's Docket No. 704660-3001  
Application No. 10/646,221

**APPENDIX E**

**U.S. Patent No. 6,605,449 to Short**

Attorney's Docket No. 704660-3001  
Application No. 10/646,221

**APPENDIX F**

**U.S. Application No. 10/646,221 as published under Publication No. 20040248253**

**APPENDIX G**

**Comparative Timeline for Short and Patten Applications**

## APPENDIX G

Short

09/594,459 6/14/00  
6,605,449 8/12/03

CIP

09/332,835 6/14/99

Patten et al.

10/646,221 8/22/03

CON

09/559,671 4/27/00  
6,613,514 9/2/03

CON

08/769,062 12/18/96  
6,335,160 1/1/02